MOTIF VATIONS

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ACTIVE MOTIF

Innovative Tools for
Cell & Molecular Biology

Simple, Efficient Analysis of ERK1/2 Phosphorylation

Fast Activated Cell-based ELISA (FACE™) Kits* provide a simple, sensitive and efficient method to monitor protein phosphorylation. FACE Kits enable you to perform modification-specific analysis directly within the cell, making them the simplest phospho-specific assay available. This also means there's no need for time-consuming cell extractions, gel electrophoresis or membrane blotting, which saves you both time and money.

The extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2) are important members of the mitogen activated protein kinase (MAPK) family of proteins that are found in all eukaryotes. The ERK1/2 signaling cascade has been shown to be a critical regulator of cell differentiation, cell physiology and neural function. Loss of regulatory control of these proteins has been implicated in a variety of pathological conditions, including cancer and autoimmune diseases. Yet, despite widespread interest in ERK1/2 function, there is a lack of sensitive, convenient assays that are suitable for modern high-throughput drug discovery research. That's why the scientists at Active Motif developed the FACE ERK1/2 Kit.

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Colorimetric Quantitation of Activated IRF Family Members

Active Motif's new TransAM™ IRF Family Kit makes it possible to rapidly profile the levels of four different IRF transcription factor family members in one simple experiment, and to compare the levels obtained under different growth and stimulation conditions. Because TransAM is an ELISA-based, colorimetric method*, it eliminates the use of radioactivity and provides quantitative results in less than five hours. This makes TransAM faster, more sensitive and simpler than other techniques, like gelshift and Western blotting.

Interdependent family

Interferon Regulatory Factors (IRFs) are a family of transcription factors that play a critical role in host defense. The many members of the IRF family are involved in the regulation of interferon α and β, which in turn regulate host immunity, cell growth and hematopoietic development. As the function and activity of individual IRFs can depend both on their activation state as well as that of other IRF family members, the ability of the TransAM IRF Family Kit to simultaneously profile the activation states of IRF-1, -2, -3 and -7 (Figure 1) is critical to understanding their role in the regulation of immune function and oncogenesis.

The TransAM method

The TransAM method facilitates the study of transcription factor activation in mammalian tissue and cell culture extracts. The TransAM IRF Family Kit utilizes a 96-well

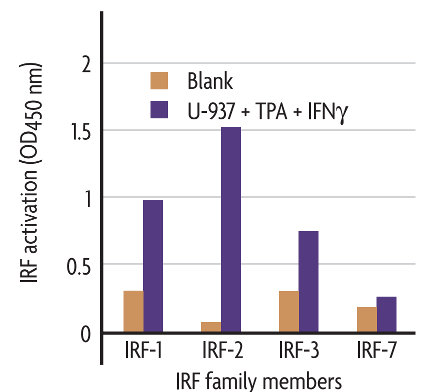

Figure 1: Profiling activation of various IRF family members. The TransAM IRF Family Kit was used to assay IRF-1, -2, -3 and -7 activation using 5 µg/well nuclear extract prepared from U-937 cells that had been cultured in medium supplemented with 5 nM TPA (phorbol, 12-myristate, 13 acetate) for 72 hours and stimulated with 5 ng/ml interferon gamma for two hours at 37°C prior to harvesting.

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Rapid, Accurate Monitoring of IκBα Phosphorylation

Active Motif's FunctionELISA™ IκBα Kit provides a fast, accurate method to monitor the phosphorylation state of IκBα. FunctionELISA Kits utilize a Sandwich ELISA technique that is faster and simpler to perform than other techniques, like Western blots. In just hours, the FunctionELISA IκBα Kit enables sensitive, quantitative measurement of phosphorylated IκBα.

IκBα is widely studied because it is an inhibitor of NFκB. In the majority of cells, NFκB exists in an inactive form in the cytoplasm, bound to the inhibitory IκB proteins. External stimuli, such as tumor necrosis factor (TNF) or other cytokines, initiate a signal transduction cascade that leads, ultimately, to the degradation of IκBα. The NFκB dimers then translocate to the nucleus and activate target genes. Thus, the FunctionELISA IκBα Kit provides an accurate means both to monitor the phosphorylation state of IκBα and to correlate this information with the activation and translocation of NFκB.

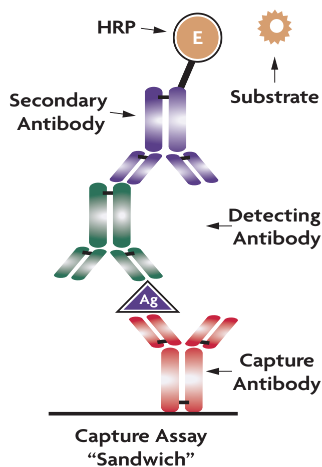

Figure 1: Sandwich ELISA schematic.

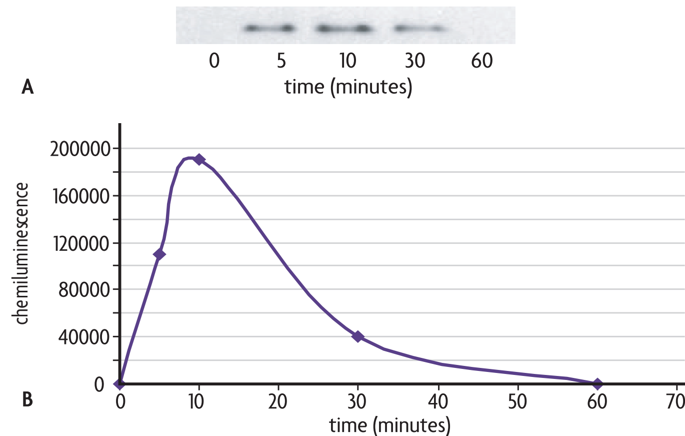

Figure 2: Induction of IκBα phosphorylation with TNF-α.
Jurkat cells were grown to 2 x 10^6 cells/ml and treated with 1 nM TNF-α. Cells were harvested at various indicated time points and cell lysates were tested in Western analysis (Fig. 2A) using an Phosphorylated-IκBα antibody (Active Motif Cat. No. 40904) and the FunctionELISA IκBα Kit (Fig. 2B).

Fast, convenient format

FunctionELISA IκBα offers a simple, rapid method to monitor the phosphorylation state of IκBα. The kit utilizes the Sandwich ELISA technique, which utilizes two antibodies directed against different epitopes to capture and quantifiably measure phosphorylated IκBα (Figure 1). Compared to Western blotting, FunctionELISA is faster and simpler to perform, as it does not require the running, blotting and developing of gels. Plus, the kit's 96-well format makes it possible to process multiple samples quickly, at whatever level of throughput is best for you.

The FunctionELISA advantage

- Analyze multiple samples in low-volume, high-throughput experiments
- Analysis complete in just hours
- Provides more quantitative results

Quantitative results

The Capture Antibody in the FunctionELISA IκBα Kit specifically binds the phosphorylated form of IκBα. A polyclonal Detecting Antibody is then used with an HRP-conjugated Secondary Antibody to measure the bound IκBα. For increased sensitivity, FunctionELISA IκBα Kits utilize a highly sensitive chemiluminescent detection method that uses a luminometer. As demonstrated, FunctionELISA provides results that are much more quantitative than those that can be generated by Western blot analysis (Figure 2).

Complete kit ensures reproducibility

FunctionELISA IκBα contains all of the reagents required to quickly quantify phosphorylated IκBα in your sample, including recombinant phospho-IκBα that can be used to generate a standard curve. For fast, accurate quantification of phosphorylated IκBα, try the FunctionELISA IκBα Kit.

Product	Format	Catalog No.
FunctionELISA™ IκBα	1 x 96-well plates 5 x 96-well plates	48005 48505
FunctionELISA™ TRAIL	1 x 96-well plates 5 x 96-well plates	48010 48510
FunctionELISA™ Cytochrome c	1 x 96-well plates 5 x 96-well plates	48006 48506

Get More Effective Gene Silencing with gripNAs™

Active Motif's custom gripNA™* oligonucleotides provide you with a better alternative for gene silencing. Traditional gene inhibition reagents such as DNA-antisense, siRNA, S-oligos and morpholinos often suffer from mistargeting due to non-specific interactions as well as from difficulties with transfection. However, gripNAs have superior sequence specificity, are resistant to nuclease degradation and offer simple delivery with the use of Active Motif's novel protein delivery reagent, Chariot™ II. This makes gripNAs the tool of choice for your gene silencing experiments.

Why gripNAs?

- Unsurpassed sequence specificity
- Resistant to nucleases
- Flexible synthesis modifications
- Highly soluble
- Easy delivery with Chariot II
- Simple online ordering

A better backbone

gripNA oligonucleotides are a novel form of negatively charged Peptide Nucleic Acids (PNAs). PNAs are DNA analogs in which the nucleoside bases are attached to a synthetic backbone rather than to deoxyribose, as in DNA. PNAs bind complementary DNA and RNA by Watson-Crick base pairing with even higher affinities than nucleic acids. PNAs can also invade and bind double-stranded DNA and are resistant to nucleases. However, poor water solubility has limited their effectiveness in gene silencing.

Improved synthesis

To overcome the limitations of traditional PNAs, Effimov *et al*, developed PNAs with negative charges[1]. The result is gripNAs, which are highly soluble and bind RNA with high affinity and sequence specificity. This reduces mistargeting and improves your gene inhibition studies.

Proven results

gripNAs are proven effective in mammalian cells, Zebrafish, *Xenopus* and in *in vitro* translation systems. To illustrate, a synthesized gripNA probe that was targeted against human cyclin B1 was delivered into human fibroblast (HS-68) cells using Chariot II. Western blot analysis performed 20 hours post-delivery clearly indicates that the cyclin B1 signal has been inhibited (Figure 1).

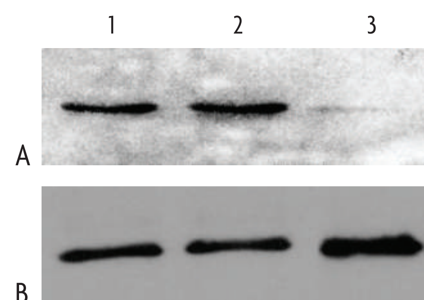

Figure 1: gripNA silencing of cyclin B1.
An 18-mer gripNA probe (1 µM) targeted against cyclin B1 was complexed with Chariot II. gripNA probe alone and the Chariot II/gripNA probe complex were overlaid onto cultured HS-68 cells synchronized by serum starvation for 40 hr, then released by addition of serum for 4 hr. Expression of cyclin B1 protein was analyzed by Western blot after 20 hr (A). Cdk2 protein was also analyzed by Western blot to normalize the amount of protein loaded in each lane (B). Data provided by Dr. L Chalion, Dr. M. Morris and Dr. G. Divita, CNRS, Montpellier, France.
Lane 1: Negative control
Lane 2: Addition of gripNA probe only
Lane 3: Addition of Chariot II/gripNA probe complex

Simple, non-cytotoxic delivery

The delivery of gene silencing reagents into the cell is a major limitation for most experiments. Classical delivery mechanisms such as microinjection and DNA/RNA transfection can be time consuming and inefficient. These conventional methods may also result in high levels of cytotoxicity within your target cells. Active Motif's Chariot II delivery reagent offers simple, efficient delivery of gripNAs with minimal cytotoxicity (Figure 2), providing you with a complete solution for gene silencing.

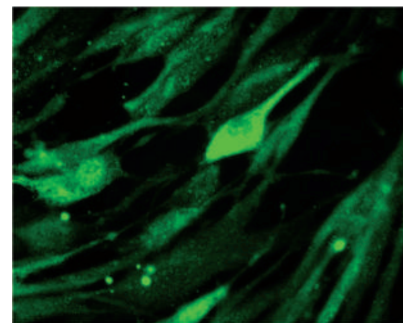

Figure 2: Chariot™ II delivery of Fluorescein-tagged gripNA.
An 18-mer gripNA probe (1 µM) labeled on its 3´ end was complexed with Chariot II in PBS and incubated for 30 min at 37°C, then overlaid onto cultured HS-68 for 1 hour. Cells were washed extensively prior to observation.

Order 24/7

All the information you need to design, order and use your gripNA probe can be found at www.activemotif.com/gripna. gripNAs can be ordered 24 hours a day, 7 days a week using our simple online ordering system. gripNA probes can be ordered with or without Chariot II. In addition, you can add a 3´ modification (Biotin, FITC or primary amine) to your probe. Each gripNA is verified by mass spectrophotometry and supplied with a fluorescently labeled positive control to ensure your success. Log on now and order your custom gripNA today.

1. Effimov *et al.* (1998) *NAR* 26: 566-575.

Product	Format	Catalog No.
Custom gripNA™ Probe	200 nmol	24001
Custom gripNA™ Probe w/Chariot™ II	200 nmol	24002
Primary Amine Modification		24004
Biotin Modification		24005
Fluorescein Modification		24006
gripNA™ Human CREB Positive Control	25 nmol	24007
Chariot™ II	96 rxns	24008

continued from page 1 — Simple, Efficient Analysis of ERK1/2 Phosphorylation

The FACE™ method

FACE is a sensitive assay designed to detect activated proteins within mammalian cells. In the FACE method (Figure 1), cells are cultured in 96-well plates and stimulated to induce the pathway of interest. Following stimulation, the cells are fixed, washed and quenched. Each well is then incubated with a primary antibody specific for the activated protein of interest. Addition of secondary HRP-conjugated antibody and developing solution provides an easily quantified, colorimetric readout. The number of cells in each well can be normalized simply with the provided Crystal Violet solution. FACE Kits are easy to use and require less than 2 hours of hands on time, giving you the freedom to accelerate your research.

The FACE advantage

- Simple, quantitative method
- Fixing cells preserves the protein's activation state
- Cell-based assay eliminates extractions and gel electrophoresis
- Increased target epitope exposure

Specificity for confident conclusions

The excellent specificity and sensitivity achieved using the FACE ERK Kit is demonstrated in Figure 2. To ensure that you are able to achieve comparable results, we test all antibodies included in FACE Kits for cross-reactivity. The included total-ERK1/2 antibody will recognize all ERK1/2 present within the cell, regardless of its phosphorylation state, whereas the phosphospecific antibody detects only ERK1/2 that is phosphorylated on Thr202 and Tyr204 or Thr185 and Tyr187 for ERK1 and ERK2, respectively. Using FACE Kits you'll achieve increased sensitivity and results you can be confident in.

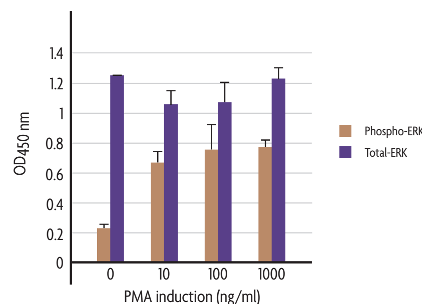

Figure 2: Measurement of phosphorylated and total ERK. Murine Macrophage 4/4 cells were cultured in 96-well plates and serum-starved for 16 hours. Cells were then stimulated with the indicated amounts of Phorbol 12-myristate 13-acetate (PMA) for 10 minutes and fixed. Total and phospho ERK were each assayed in triplicate using the phospho and total ERK antibodies included in the FACE ERK1/2 Kit. Data was plotted after correction for cell number (performed through use of Crystal Violet). Note that the induction treatment did not affect the overall level of total ERK.

Instant fixation for accurate results

Preparation of cellular extracts prior to Western blotting or ELISA analysis takes at least one hour to perform. In addition, Western analysis requires the determination of protein concentration prior to loading your gel. During this time, additional protein modifications can occur, which will alter your results. FACE Kits, however, use a fixation step that "freezes" the cellular state. This means that there is no risk of further protein modification before analysis, and gives you a clear view of the protein state within the cell at that specific time point. Improve the accuracy of your results by using FACE.

FACE Kits are also available for monitoring the cellular levels of phosphorylated p38, JNK and AKT, with many other kits to be released throughout 2003. For a better way to monitor protein phosphorylation, try FACE.

* Developed in agreement with Dr. M. Peppelenbosch and Dr. H. Versteeg.

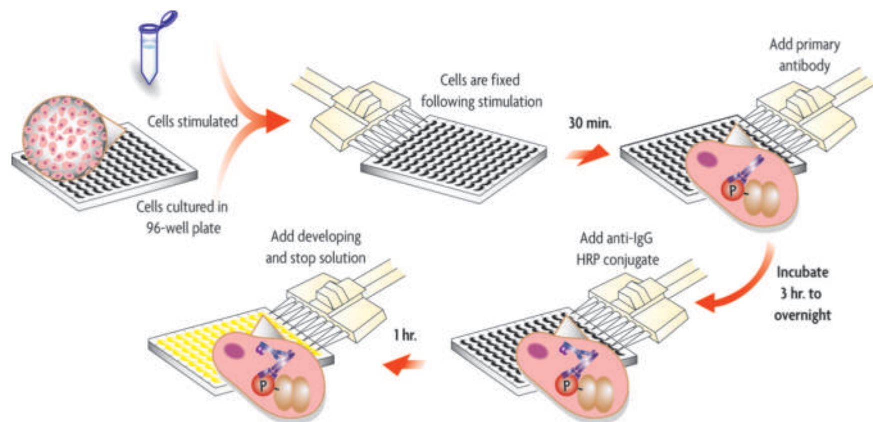

Figure 1: Flowchart of the FACE procedure.

Product	Format	Catalog No.
FACE™ ERK1/2	1 x 96 rxns 5 x 96 rxns	48140 48640
FACE™AKT	1 x 96 rxns 5 x 96 rxns	48120 48620
FACE™ JNK	1 x 96 rxns 5 x 96 rxns	48110 48610
FACE™ p38	1 x 96 rxns 5 x 96 rxns	48100 48600

continued from page 1 — Colorimetric Quantitation of Activated IRF Family Members

plate containing double-stranded oligonucleotide that includes the consensus-binding site for IRF. When nuclear or whole-cell extract is added, the various members of the IRF family bind to the oligonucleotide. Primary antibodies that are specific for the activated forms of each IRF family member are then added to individual wells, followed by incubation with secondary HRP-conjugated antibody and developing reagent. Reading the plate on a spectrophotometer provides a quantitative, colorimetric readout (Figure 2). Thus, the TransAM IRF Family Kit makes it possible to rapidly profile the levels of each family member under various growth and stimulation conditions (Figure 1, Page 1).

Improved method

TransAM assays are a marked improvement over other techniques used to study transcription factor activation. Unlike gelshift, Western blotting and reporter gene methods, TransAM does not use inefficient cloning and cell transfections, time-consuming gel exposures or radioactive probes. Inconsistencies due to variable reporter plasmid transfection and the need to construct stable cell lines are also eliminated. TransAM assays are complete in mere hours, rather than days, are more sensitive and provide quantitative results. Moreover, TransAM can be used to study stimulated tissue samples.

The TransAM advantage

- Non-radioactive, colorimetric method provides quantifiable results
- Results in less than 5 hours
- 10-fold greater sensitivity than gelshift
- Simultaneous profiling of multiple family members
- Ability to assay cells or tissue samples

A family of families

In addition to the new TransAM IRF Family Kit, Active Motif offers TransAM Family Kits for simultaneously studying members of the AP-1, NFκB and STAT transcription factor families. TransAM Kits for monitoring the individual transcription factors NFκB** p50 and p65, HIF-1, c-Fos, FosB, c-Jun, PPARγ, p53, MyoD, Sp1, Sp3, C/EBP, NF-YA, ER, STAT3, NFATc1 and total and phosphorylated CREB are also available. TransAM Chemi Kits that are 100-fold more sensitive than gelshift assays are available for NFκB p50 and p65. For a better method to study transcription factor activation, try TransAM Kits.

* Technology covered under AAT-filed patents and licensed exclusively to Active Motif.

** Use of TransAM in NFκB-related drug discovery may be covered under U.S. Patent No. 6,150,090 and require a license from Ariad Pharmaceuticals (Cambridge, MA, USA).

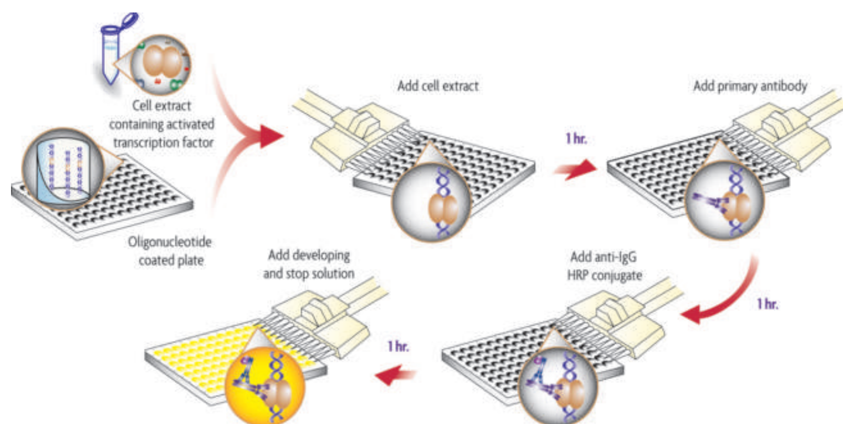

Figure 2: Flowchart of the TransAM procedure.

Product	Format	Catalog No.
TransAM™ IRF Family	2 x 96-well plates	45296

BIOBASE - The TRANSFAC® Company

BIOBASE is the leading content provider for gene regulation. Its industry standard databases, TRANSFAC® and TRANSPATH®, provide expert knowledge for data analysis and utilization. With long-standing experience in gene regulation, BIOBASE offers researchers not only database subscriptions, but also scientific consulting. Over 100 years of scientific excellence in this domain are accumulated at BIOBASE to solve your problems, royalty-free.

TRANSFAC – Transcription factor database

With over 25,000 entries, BIOBASE's TRANSFAC is the Gold Standard of transcription factor databases. Curated by biologists, TRANSFAC is comprised of data from approximately 10,000 publications. Divided into two main sections, TRANSFAC provides knowledge about the proteins and their binding sites. With an easy-to-use, web-based user interface, TRANSFAC offers encyclopedic knowledge in a compressed form, as well as tools for in-depth analysis of sequences and gene expression data.

TRANSPLORER™ – Binding site elucidation

The Java application TRANSPLORER™ enables complete analysis of regulatory DNA sequences. The integrated Dragon Promoter Finder and Dragon Gene Start Finder (from the Laboratories for Information Technology, Singapore) are used to predict promoter regions in genomic sequences. TRANSPLORER's powerful matrix prediction engine allows immediate promoter analysis. Tightly linked with TRANSFAC, TRANSPLORER is a comprehensive toolbox that enables the scientist to analyze and understand a gene's regulation.

For more information, visit BIOBASE's website at http://www.biobase.de or contact their marketing department by email at marketing@biobase.de or by phone at +49 (5331) 85 84 30.

Efficient Protein Transfection using Chariot™

Chariot™ is a revolutionary protein delivery reagent that efficiently transports biologically active proteins, peptides and antibodies directly into cultured mammalian cells in less than two hours. The cells can be assayed immediately after delivery to determine the effects of the introduced material, without the need for fixing. This makes Chariot ideal for functional studies in living cells.

? How does Chariot work?

Chariot forms a non-covalent complex when combined with a purified protein, peptide or antibody. This complex stabilizes the macromolecule, protecting it from degradation during internalization. It also eliminates the need for fusion proteins or chemical coupling. After delivery, the complex dissociates in a process called "decaging", leaving the macromolecule biologically active and free to proceed to its cellular target. The delivery process is serum independent, giving you the flexibility to culture your cells in whichever media type you prefer.

? Does Chariot work with primary cells or even plant cells?

Chariot delivers effectively into primary cells, something that most other delivery systems cannot do. Most notable is the use of Chariot on hard to transfect primary neurons (Jurney W. *et al. Journal of Neuroscience* 22: 6019-6028. 2002) without any signs of cytotoxicity or rejection. Chariot has also been shown to deliver proteins into plant protoplasts (Wu, Yan, *et al*. *The Plant Journal*. 33: 131-137. 2002).

? What about protein functionality?

The ability of Chariot to deliver biologically active protein is shown using a 119 kDa subunit of β-galactosidase. β-galactosidase is composed of four subunits that must assemble to form the functional protein. HeLa cells turn blue upon addition of X-gal following delivery of the Chariot-galactosidase complex, demonstrating successful delivery of functional β-galactosidase (Figure 1).

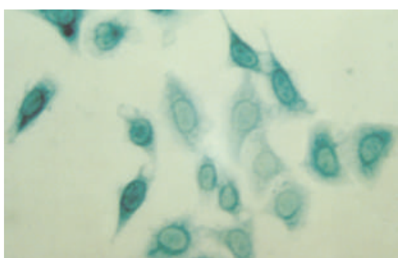

Figure 1: Chariot delivery of β-galactosidase.
One μg of a 119 kDa subunit of β-galactosidase was complexed with Chariot™ Protein Delivery Reagent (see page 6) for 30 minutes and delivered into HeLa cells. Cells were fixed and stained with X-gal 2 hours post-delivery.

? What are researchers using Chariot for?

We have an ever growing list of citations from researchers who have used Chariot in their published research. Chariot is becoming the number one choice for protein, peptide and antibody delivery into both primary and cultured cells. Peluso *et al* transfected a peptide and an antibody into granulosa cells to study the PKCd-Dependent Pathway (Endocrinology. Vol. 142(10): 4203-4211. 2001).

Product	Format	Catalog No.
Chariot™	25 rxns 100 rxns	30025 30100

Another recent publication shows the usage of Chariot in chick ganglions to study the coordinated activation of autophosphorylation sites in RET Receptor Tyrosine Kinase (Coupier *et al*, *JBC*. Vol. 277, No. 3. 2002. 1991-1999). And Ikari *et al* used Chariot to study up-regulation of Sodium-dependent glucose transporter by interaction with Heat Shock protein 70 in epithelial cells (*JBC*. Vol. 277, No. 5. 33338-33343. 2002).

Log on to our website at www.activemotif.com to download the protocol as well as the most current list of publications that cite Chariot.

? Is there any size limit to the proteins that Chariot can deliver?

There is one limitation; peptides below 12 amino acids are too small for Chariot to efficiently deliver. However, we have not found a maximum size limitation. The key to remember is that the larger the protein or antibody, the longer it takes to get in; but it will get in.

? Does Chariot delivery use the endosomal pathway?

No, Chariot delivery is independent of the endosomal pathway. Temperature studies have been performed at 37°C and at 4°C with almost identical results. This demonstrates that Chariot is independent of the endosomal pathway as it is shut down at 4°C.

? What is the difference between Chariot and Chariot II?

Chariot is intended for peptide, protein and antibody delivery. New this year is Chariot II, which is for gripNA delivery. Chariot II has modifications that are optimized for use in transfecting gripNAs for gene silencing. To learn more about gene silencing with gripNAs, turn to page 3 in this newsletter.

Faster, More Accurate Measurement of NO Production

The Nitric Oxide Quantitation Kit is a faster, more sensitive method for measuring the production of nitric oxide (NO) in your samples. The kit employs an innovative cofactor technology that reduces the time and number of steps needed to measure NO levels. Plus, the Nitric Oxide Quantitation Kit has a wider dynamic range of NO measurement than conventional technology, giving you more accurate results. And, the kit can be used with a large variety of sample types, including plasma, serum, saliva, urine, cell lysate, tissue homogenate and tissue culture medium.

NO is a key molecule that, either directly or through intracellular signaling, stimulates host defenses in the immune system, maintains blood pressure in the cardiovascular system and modulates neural transmission in the brain. Production of NO by Nitric Oxide Synthase (NOS) can be regulated by physiological changes in intracellular calcium concentrations or induced in a cell-specific manner. Monitoring of NO production in cells is a useful tool for determining NOS activity. However, the extremely short half-life of NOS makes it unsuitable for detection.

The old way

The best index of total NO production is the sum of both the nitrite and nitrate, commonly quantified in a Two-Step assay. The first step is the conversion of nitrate to nitrite by the use of NADH or NADPH-dependent nitrate reductase. Nitrite levels are measured by the addition of Griess Reagent, which converts nitrite into a purple-colored azo compound. NO concentration is then indirectly measured using spectrophotometry (Figure 1). However, assay sensitivity is limited when using the Two-Step method because excess NADPH, which is an essential cofactor in step one, interferes with the Griess Reagent in step two. Assay sensitivity can be increased through the addition of a third step, whereby lactate dehydrogenase (LDH) eliminates excess NADPH prior to the Griess reaction. However, while inclusion of a third LDH step increases sensitivity, it is not optimal as it also increases the time required to perform each assay.

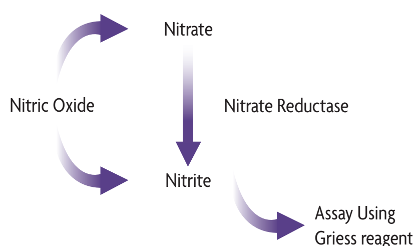

Figure 1: Measurment of NO by Griess Reagent.
Nitric oxide is converted to nitrite, then assayed using Griess Reagent.

Save time and money

The Nitric Oxide Quantitation Kit offers a faster and easier-to-use alternative to conventional Two- and Three-Step NO assays. Active Motif's Nitric Oxide Quantitation Kit contains a unique formulation of cofactors that accelerate the conversion of nitrate to nitrite, while simultaneously degrading NADPH. The result is that the time required to perform the reductase step is decreased to only 30 minutes and there's no need for a complicated, time-consuming third step, which saves you both time and effort.

Improved accuracy for better results

In addition to its increased speed and user friendliness, the Nitric Oxide Quantitation Kit provides more accurate measurement over a wider range of sample concentration than possible with other currently used methods. The improved linearity over a broader dynamic range (Figure 2) means that your results will be more precise with less optimization and repetition than is possible with any other kit.

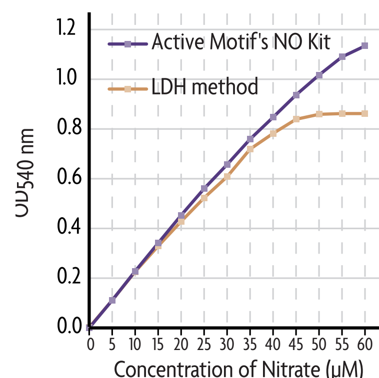

Figure 2: Dynamic range of nitrate standard curves.
Nitrate standard curves produced using the Nitric Oxide Quantitation Kit and a conventional Three-Step LDH-method kit.

Get better results today

The Nitric Oxide Quantitation Kit makes NO measurement more sensitive, faster and easier to perform than other, more conventional methods. In addition, its 96-well format enables straightforward high-throughput automation. For a better method of measuring NO production, try the Nitric Oxide Quantitation Kit today!

Product	Format	Catalog No.
Nitric Oxide Quantitation Kit	2 x 96 rxns	40020

More Specific 6xHis Protein Purification using Silica Resin

Active Motif's Ni-TED Protein Purification System is a versatile and powerful tool for isolating recombinant 6xHis-tagged proteins. Unlike traditional resins made of agarose, Ni-TED uses a silica resin that makes purification of 6xHis proteins better than ever before.

Increased specificity

Conventional protein purification using agarose-based nickel columns is often characterized by co-purification of untagged proteins due to non-specific binding to the agarose matrix. Ni-TED's silica resin has fewer side chains than agarose, which increases the selectivity of Ni-TED and reduces the non-specific binding of cellular proteins. This results in a much cleaner purification of your target protein (Figure 1).

Quick and easy

Ni-TED uses a simple one-step protocol that is ideal for purifying 6xHis-tagged proteins produced in any expression system. You can choose to elute your proteins under both native and denaturing conditions by using either competition with imidazole or a simple reduction in pH.

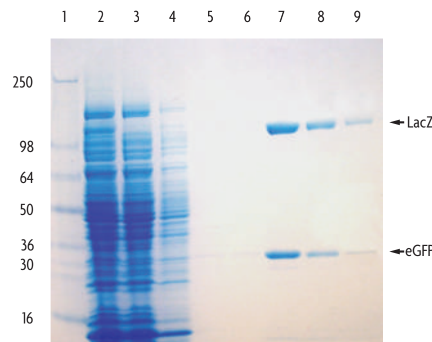

Figure 1: Dual expression in the Cascade™ Expression System.
LacZ and eGFP were cloned into pCHROMO and pCHROMO2, respectively, and integrated into TAP *E. coli*. Five hours after induction (2 mM salicylate), the cells were lysed and co-purification was performed using Ni-TED Silica, then run on a 4-20% Tris-Glycine gel.

Lane 1: Protein standard (kDa)
Lane 2: Lysate
Lane 3: Flow-through
Lanes 4-5: Washes
Lanes 6-9: Elutions

Flexible formats

Ni-TED comes in a variety of formats to meet your purification needs. For low-volume elutions in small-scale protein isolations, Ni-TED Spin Columns feature a silica-based membrane with a binding capacity of 50 µg that is optimized for use with proteins or peptides of < 100 kDa. These Ni-TED Spin Columns are conveniently available in packages of 25 or 100 with or without Lysis, Wash and Elution Buffers. To meet your large-scale needs, Ni-TED Silica is available in prepackaged columns or in bulk for purifications from culture volumes of up to 100 ml. The Ni-TED silica resin will bind up to 3 mg of His-tagged protein per gram and is suitable for isolating proteins of any size. For quick, efficient purification you can count on, try Ni-TED today.

Product	Format	Catalog No.
Ni-TED™ Spin Columns	25 rxns 100 rxns	97025 97100
Ni-TED™ Spin Columns (w/solutions)	25 rxns 100 rxns	98025 98100
Ni-TED™ Silica (0.3 g resin in prepacked columns)	5 columns 20 columns	97005 97020
Ni-TED™ Silica	1 g	97001

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